Characterizing stoichiometry and kinetics of two thermophilic nitrification communities: a crucial step in the development of thermophilic biotechnology for nitrogen removal

Tom G.L. Vandekerckhove[1], Samuel Bodé[2], Chaïm Demulder3, Nico Boon[1,*] and Siegfried E. Vlaeminck[1,4*]

[1] Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, 9000 Gent, Belgium

[2] Isotope Bioscience Laboratory, Ghent University, Coupure Links 653, 9000 Gent, Belgium

3 BIOMATH, Department of Mathematical Modelling, Statistics and Bioinformatics, Ghent University, Coupure Links 653, 9000 Gent, Belgium

[4] Research Group of Sustainable Energy, Air and Water Technology, University of Antwerp, Groenenborgerlaan 171, 2020 Antwerpen, Belgium

*These authors contributed equally and are both senior authors for this work

In several biological nitrogen removal applications, thermophilic treatment has the potential to be more cost-effective than its mesophilic counterpart. The main challenge in the development of this novel process is knowhow on how to establish thermophilic nitrification. Recently, two strategies were successful in obtaining two bioreactor communities nitrifying at 50°C. The one being achieved from a constant high temperature operation inoculated with compost material (‘T-constant reactor’), the other from mesophilic biomass being subjected to gradually increasing temperatures (‘T-increase reactor’). Most dominant nitrifiers were ammonia oxidizing archaea (AOA) of the *Nitrososphaera* genus and nitrite oxidizing bacteria (NOB) belonging to *Nitrospira,* yet the respective functional phylotypes of both reactors revealed distinctly different players. This study extensively compared the nitrification functionality of both communities. A novel method was used to determine biomass yield, monitoring the incorporation of ^{13}C during nitrogen oxidation. Similar values were obtained for AOA (0.22±0.01 vs. 0.19±0.01 g VSS g^{-1} N) and NOB (0.025±0.002 vs. 0.027±0.008 g VSS g^{-1} N). Ammonia oxidation rates were also comparable for both, reaching up to 216±18 mg N g^{-1} VSS d^{-1}, while NOB activity in the T-constant reactor was slightly lower than in the T-increase reactor (204±23 vs. 283±33 mg N g^{-1} VSS d^{-1}). The communities were further characterized by high substrate affinity for ammonium (0.12 vs. 0.32 mg N L^{-1}), nitrite (0.68 vs. 0.27 mg N L^{-1}) and oxygen (0.08 and 0.15 mg O_2 L^{-1} for AOA and NOB of T-increase reactor). Compared to NOB, AOA were less susceptible to salt stress, far less vulnerable to nitrite/free nitrous acid, but had a more narrow pH optimum and were more sensitive to ammonium/free ammonia. Although the strategy used rendered different communities, the functionality and stoichiometry of both showed only minor differences. Overall, with these insights, the challenge in the development of thermophilic nitrogen removal can now shift towards combining nitrification with anoxic processes.